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SEMI-ANNUAL PROGRESS REPORT

Report Prepared by Dr. R.E. Kallio

Date: July 19, 1952

For Period: January 1, 1952
to July 31, 1952

Authority - Contract NR 135-061 - 2/29/52

Annual Rate: $5,000

Contractor: State University of Iowa

Principal Investigator: R.E. Kallio, Assistant Professor of Bacteriology

Assistant: A.D. Larson, M.S., Research Fellow
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Title of Project: Study on Microbial Physiology; Properties and Characterization of Bacterial Urease

Objectives: 1. Purification and crystallization of bacterial urease from a highly ureolytic organism and the establishment of the role of this system in the vital economy of the cell.

2. Physico-chemical characterization of the enzyme.

3. Enzymatic, physico-chemical and immunological comparisons of the bacterial enzyme with the urease of the jack bean.

Abstract of results:

A. Since start of project

1. A medium which has proved excellent for Bacillus pasteurii (U.S.D.A. No. 673) for both total yield of organisms and activity is composed of nutrient broth, 0.5% yeast extract and 2% urea. This medium gives total crop yields of three to four times that of nutrient broth and meat infusion broth mixtures. Since growth on nutrient broth plus 2% urea is minimal the yeast extract evidently furnishes essential metabolites. Manometric CO_2 determinations of ureolytic activity indicate that the production of urease is constant as long as the organisms are grown in the presence of urea and is apparently independent of other factors. Cells grown in the absence of urea or in a medium containing ammonium salts at high pH values have proven unsatisfactory because of poor crop yields and low enzymatic activity.

2. Of sixteen urea derivatives tested only urea nitrate (which hydrolyses to urea) was attacked by resting cells of Bacillus pasteurii. The optimal pH for resting cells is 5.8 to 6.2. In the case of the cell extract the optimum pH is 6.4 to 6.6.

3. By means of new fractionation procedures involving the use of protamine for the removal of nucleic acids and adsorption and elution of the urease on calcium phosphate gel followed by fractionation with acetone and ammonium sulfate the ureolytic activity has been increased to 180,000 Sumner units/gram of bacterial protein (this activity is significantly higher than the activity of crystalline jack bean urease). This is an increase in specific activity of 50 to 60 times that of the crude starting material. However, attempts to crystallize the enzyme from acetone or ammonium sulfate have been unsuccessful. The finding that the frozen cell extract will keep for long periods of time without loss in activity should facilitate working with larger batches and make further fractionating possible.

4. For purposes of comparison jack bean urease has been prepared in the crystalline state and jack bean urease antiserum has been prepared in rabbits. If the bacterial urease can be purified to a point of where it is homogeneous protein then anti-enzyme activities will be compared.

5. The fact that urea serves as an ammonium source for growth better than ammonium salts indicates that urea serves a function in the cell economy above and beyond that of a nitrogen source. Since growth in a 2% urea medium results in extensive liberation of gaseous ammonia and a final pH of the growth medium of ca 9.2 it was thought that urea served three purposes: first, a constant and renewed supply of ammonium for cell growth; second, the regulation of the pH of the medium; and third, the furnishing of carbon dioxide. However, experiments designed to test this premise by supplying the organisms with equivalent amounts of NH_3 and CO_2 resulted in no growth. Controls using a medium containing 2% urease gave the usual heavy growth. Arginine will not replace urea or ammonium salts.

6. Warburg studies using whole cells have been carried out to determine the energy source of Bacillus pasteurii. No oxygen uptake has been obtained with eight of the sugars, with acids of the tricarboxylic acid cycle, or with fatty acids. Certain amino acids are oxidised at pH values from 8 to 9, serine, glutamic and alanine being oxidised at the most rapid rates. These studies indicate that amino acids serve as the main energy supplying compounds. The cell extracts contain an active aspartic-α-ketoglutaric transaminase. This raises the question as to why α-keto acids are not oxidized. Further work is in progress along these lines.

B. During current report period

These periods coincide.

Plans for the future

A. Immediate

1. Since reasonable success has been obtained on the purification of urease, but crystallization attempts have failed, electrophoretic analysis of the purest fractions will be done. This will indicate whether further purification is necessary before crystallization or whether new methods for crystallising bacterial urease must be developed. Whatever the results of electrophoresis are, further attempts will be made toward the goal of purification and crystallization of the enzyme.

2. Standard kenetic studies will be carried out on the purified enzyme. Studies on the composition of urease are also planned. These will consider the number of sulfhydryl groups, action of inhibitors, and various physico-chemical tests all in comparison with plant urease.

3. Further studies are intended to determine the function of urea in the economy of the cells metabolism. A further insight may be gained by Warburg studies on various keto acids plus ammonia or urea since amino acids are oxidised.

B. Future - long range

1. Cell extracts will be used to study the amino acid metabolism of the organism. The activities of transaminase and decarboxylase using various amino acids will be determined, and also the utilization of α-keto acids will be ascertained. These data should give some insight as to the physiology of the organism.

2. Genetic analysis of the bacterium in relationship to mycolytic activity has been impeded because of the difficulty in obtaining accurate plate counts. As suitable methods are found for this type of study the genetic analysis will be extended.

Reports and Publications (During current report period)

It is strongly felt that in a study of this type only a complete phase of the investigation is worthy of publication.

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